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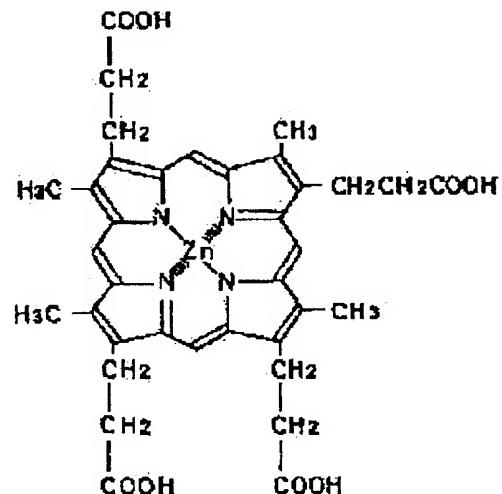
(54) THERAPEUTIC AGENT OR DIAGNOSTIC AGNET FOR MALIGNANT TUMOR AND PRODUCTION OF SUBSTANCE AC8007

(57)Abstract:

PURPOSE: To obtain a substance AC8007 useful as a therapeutic agent having properties as a photosensitizer for photochemotherapy or as a diagnostic agent for malignant tumors.

CONSTITUTION: The objective therapeutic agent or diagnostic agent for malignant tumors comprises a substance AC8007, having an estimated chemical structural formula expressed by the formula and the following physico-chemical properties or its nontoxic salt as an active ingredient:

(1) Elementary analytical value; C, 60%; H, 5%; N, 8% and Zn, 8-10%. (2) Mass spectrometric value; 717 [MH⁺, according to the fast atom bombardment-mass spectrometry (FAB- MS)]. (3) Molecular formula; C₃₆H₃₆O₈N₄Zn. (4) Solubility in solvents; soluble in methanol, ethyl acetate, acetic acid and dimethyl sulfoxide and insoluble in water, hexane and benzene. (5) Color reaction; positive to the potassium permanganate reaction and iodine reaction and negative to ferric chloride reaction, the Dragendorff reaction and ninhydrin reaction. This compound is obtained by culturing a microorganism, belonging to the genus *Arthrobacter* and capable of producing the substance AC8007, e.g. *Arthrobacter*.sp. TM-1 (FERM BP-3646) in a culture medium and then collecting the substance from the resultant culture.



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(54)【発明の名称】 悪性腫瘍の治療剤または診断剤およびAC8007物質の製造法

(57)【要約】 (修正有)

【目的】 AC8007物質またはその非毒性塩を含む
悪性腫瘍の治療剤、診断剤の提供。

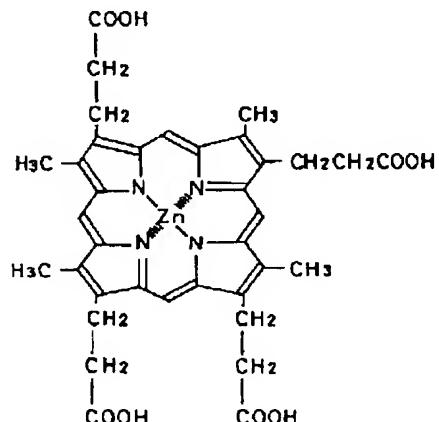
【構成】 以下の理化学的性質を有するAC8007物
質またはその非毒性塩を含む医薬。

(1) 元素分析値: C: 約60%、H: 5%、N: 約8
%、Zn: 約8~10%

(2) 質量分析値: 717 (MH⁺、FAB-MSによ
る)

(3) 分子式: C₁₆H₁₆O₈N₄Zn

AC8007物質は下記推定構造式を有し、



アースロバクター属に属するAC8007物質生産菌を
培地に培養し、培養物よりAC8007特質を採取する
ことにより製造する。

【特許請求の範囲】

【請求項1】 以下の理化学的性質を有するAC8007物質またはその非毒性塩を有効成分とすることを特徴とする悪性腫瘍の治療剤または診断剤。

(1) 元素分析値

C: 約60%、H: 約5%、N: 約8%、Zn: 約8~10%

(2) 質量分析値

717 (MH⁺, FAB-MSによる)

(3) 分子式

*10

λ_{max} 0.1N HCl・メタノール nm (E_{1cm} 1%):

少なくとも386(肩) (1275)、402(4690)、560(175)、591(60) nm付近に特徴的な吸収を有する

(5) 赤外線吸収スペクトル (KBr法)

少なくとも3420、2920、1705、1400、1275、1130、940、835 cm⁻¹付近に特徴的な吸収を有する

(6) 溶剤に対する溶解性

メタノール、酢酸エチル、酢酸、ジメチルスルホキシドに可溶性、水、ヘキサン、ベンゼンに不溶性

(7) 呈色反応

過マンガン酸カリウム反応、ヨード反応は陽性、塩化第二鉄反応、ドラーゲンドルフ反応、ニンヒドリン反応は陰性

(8) 塩基性、酸性、中性の区別

酸性物質

(9) 物質の色

暗赤色

【請求項2】 AC8007物質が下記の推定化学構造式を有することを特徴とする請求項1記載の悪性腫瘍の治療剤または診断剤。

【化3】

* C₃₆H₁₆O₈ N₄ Zn

(4) 可視部吸収スペクトル

【化1】

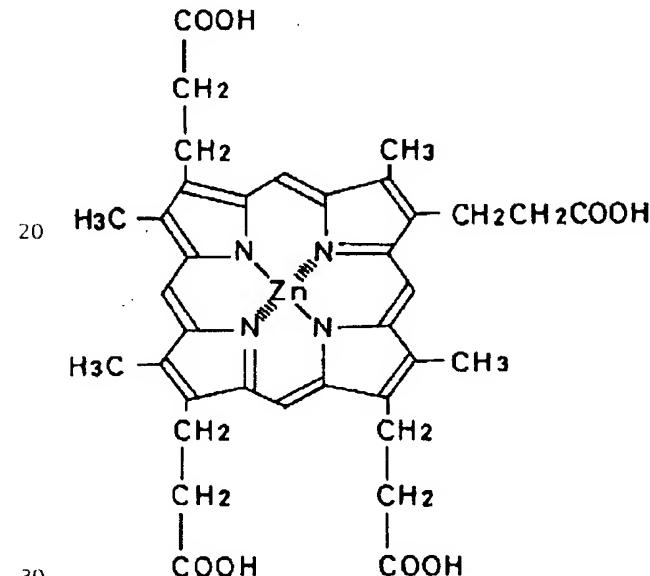
λ_{max} メタノール nm (E_{1cm} 1%):

少なくとも386(肩) (750)、406(3525)、538(185)、574(190) nm付近に特徴的な吸収を有する

【化2】

*10

λ_{max} 0.1N HCl・メタノール nm (E_{1cm} 1%):



【請求項3】 アースロバクター属に属するAC8007物質生産菌を培地に培養し、次いで培養物よりAC8007物質を採取することを特徴とするAC8007物質の製造法。

【請求項4】 アースロバクター属に属するAC8007物質生産菌が、アースロバクター・エスピード・TM-1 (FERM BP 3676) である請求項3記載の製造法。

【発明の詳細な説明】

40 【0001】

【産業上の利用分野】 本発明は、AC8007物質またはその非毒性塩を有効成分とする悪性腫瘍の治療剤または診断剤およびAC8007物質の製造法に関する。

【0002】

【従来の技術】 悪性腫瘍への光化学療法 (Photodynamic therapy: PDT) が開発されて数十年を経過し、多数の有効例が確認され、早期悪性腫瘍の根治治療あるいは診断剤として使用されている。このPDTに使用される代表的光増感剤はポルフィリン誘導体である。

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【0003】しかしながら、ポルフィリン誘導体は次のような欠点がある。(1) 化学的に単一でない、(2) 組織透過性の良い長波領域に吸収を持たない、(3) 腫瘍組織に長く保持されない、(4) 正常細胞からの排泄が遅い、(5) 光化学反応収率が低いなどがあげられる。

【0004】

【発明が解決しようとする課題】以上のような問題を一点でも解決できる光増感剤の開発は、将来のPDTに有効であると期待される。そして、副作用の少ない新規かつ有用な光増感剤が悪性腫瘍治療のために求められている。本発明の目的は、AC8007物質またはその非毒性塩を有効成分とする悪性腫瘍の治療剤または診断剤およびAC8007物質の製造法を提供するにある。

【0005】

【課題を解決するための手段】これまでのPDTにおける光増感剤は、すべて化学的に合成された物質であり、副作用の面で問題があつた。本発明者らは微生物が生産する天然の生理活性物質の内から、アースロバクター・エスピード・TM-1(微生物研究会第3676号)の培養液からAC8007物質(特開平2-234688号公報参照)と同定される物質を採取し、本物質が亜鉛を有する物質であることを見出した。更に、本発明者らは、本物質が悪性腫瘍のPDTの光感剤としての性質を*

入 \max 0.1N HCl・メタノール nm (E 1% 1cm):

少なくとも386(肩)(1275)、402(469)、560(175)、591(60) nm付近に特徴的な吸収を有する。

【0009】(5) 赤外線吸収スペクトル(KBr法): 図3

少なくとも3420、2920、1705、1400、1275、1130、940、835 cm^{-1} 付近に特徴的な吸収を有する。

(6) 溶剤に対する溶解性

メタノール、酢酸エチル、酢酸、ジメチルスルホキシドに可溶性、水、ヘキサン、ベンゼンに不溶性

【0010】(7) 呈色反応

過マンガン酸カリウム反応、ヨード反応は陽性、塩化第二鉄反応、ドーラゲンドルフ反応、ニンヒドリン反応は陰性

(8) 塩基性、酸性、中性の区別

酸性物質

(9) 物質の色

暗赤色

(10) ^1H -NMR(400MHz, 27°C, d₆-DMSO中で測定): 図4

【0011】(11) ^{13}C -NMR(100MHz, 27°C, DMSO中で測定): 少なくとも下記に示したシ

* 有する治療剤として、又、診断剤として有用であることを見出し、さらに、AC8007物質の良好な製造法を見出し、本発明を完成した。

【0006】本発明の有効物質であるAC8007物質は、少なくとも次に示すような理化学的性質を有する。

(1) 元素分析

C: 約60%、H: 約5%、N: 約8%、Zn: 約8~10%

10 (2) 質量分析 717(MH⁺), FAB-MSによる

(3) 分子式

C₃₈H₃₆O₈N₄Zn

(4) 可視部吸収スペクトル: 図1(中性条件)、図2(酸性条件)

【0007】

【化4】

入 メタノール \max nm (E 1% 1cm):

20 少なくとも386(肩)(750)、406(352)、538(185)、574(190) nm付近に特徴的な吸収を有する

【0008】

【化5】

入 0.1N HCl・メタノール \max nm (E 1% 1cm):

30 グナルが認められた。174、20(s)、147.62(s)、147.54(s)、146.83(s)、146.77(s)、146.73(s)、139.44(s)、139.30(s)、136.65(s)、136.54(s)、97.06(d)、96.95(d)、37.41(t)、21.63(t)、21.59(t)、11.46(q)

【0012】(12) 薄層クロマトグラフィー(東京化成社製、スポットフィルムシリカゲルf使用)

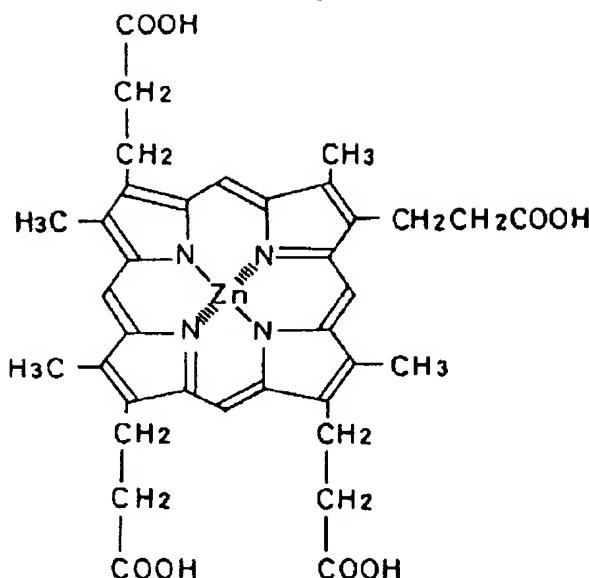
Rf = 0.45 [展開溶媒: クロロホルム-メタノール-酢酸(10:1.5:0.1)]

Rf = 0.37 [展開溶媒: ブタノール-エタノール-クロロホルム-アンモニア水(4:5:2:4)]

【0013】AC8007物質は、以上の性質を有することから、次式で表される構造であると推定される。

【0014】

【化6】



【0015】本発明の有効成分であるAC8007物質を生産するには、微生物の培養による醸酵法が最も適切である。生産に好適な微生物としては、アースロバクタ…エスピ…TM-1 (Arthrobacter sp. TM-1: 微研条寄第3676号) を挙げることができる。本菌は、滋賀県甲賀郡甲賀町の白菜畑の土壤より分離した細菌TM-1株であり、本発明に最も有効に使用される菌株の一例であって、本菌株の菌学的性質を示すと次の通りである。

【0016】尚、本菌株の同定に当たって、同定試験は「医学細菌同定の手引き、第2版、1974」や、「Microbiological Methods 3巻」等に準じて実施した。実験結果を、「医学細菌同定*

5. 生理・化学的性状

グラム染色	+
KOH反応	-
抗酸性染色	--
カプセル形成	-

【0020】

OFテスト (Hugh-Leifson)	NT
OFテスト (N源にNH ₄ , H ₂ , PO ₄)	O
好気での生育	+
嫌気での生育	+
生育温度 42°C	-
37°C	+
20°C	+
10°C	NT

【0021】

食塩耐性 0%	+
0.5%	+
3.0%	NT
5.0%	NT
生育pH 4.7	-

*の手引き、第2版、1974) 「Bergery's Manual of Systematic Bacteriology Vol. 1 (1984), Vol. 2 (1986), Vol. 3 (1989)」等に対比して同定した。培養温度は28~30°Cで行った。(+ : 陽性、(+) : 弱陽性、- : 陰性、NT : 未試験、ND : 文献に記載が無い、NC : 変化しない)

【0017】実験結果

1. 生育の特徴

10 普通寒天斜面培地

周辺はギザギザ状の丸い集落を形成し、中央が凸状に盛り上がる。半透明、湿潤で灰白色~淡黄土色を呈するが、可溶性色素は産生しない。

普通寒天平面培地

生育は、悪いが線状に生育する。半透明、湿潤で灰白色~淡黄土色を呈するが、可溶性色素は産生しない。

液体培地 (ペプトン水)

一様に混濁する。

リトモスミルク培地

アルカリになりペプトン化する。

【0018】2. DNAのGC mol %

NT

3. 主たるイソブレノイドキノン

NT

4. 形態の特徴

培養前期には長桿状を示し、彎曲し、大きさは0.8×4~5 μmでV字状を示すものもある。培養後期には1.2×1.5 μmの短桿状~球状に変化する細菌。

【0019】

7

5. 6
9. 0
10. 0

8

+

+

-

【0022】

ゲラチン分解	-
デンプン分解	-
カゼイン分解	+
エスクリン分解	NT
セルロース分解	-
チロシン分解	NT
Tween 80 分解	NT
アルギニン分解	NT
カタラーゼ産生	-
オキシダーゼ産生	NT

【0023】

レシチナーゼ産生	-
ウレアーゼ産生 (SSR)	NT
ウレアーゼ産生 (Chrys.)	NT
インドール産生	-
硫化水素産生 (lead acetate paper)	+
アセトイン産生 (K ₂ HPO ₄)	-
アセトイン産生 (NaCl)	-
MRテスト	-
硝酸塩還元テスト (ガス産生)	-
(NO ₂ ⁻ の検出)	-
(NO ₃ ⁻ の検出)	+

【0024】

シモンズ培地での利用性 (アルカリ産生)	-
クエン酸塩	-
リンゴ酸塩	+
マレイン酸塩	-
マロン酸塩	-
プロピオン酸塩	-
グルコン酸塩	(+)
コハク酸塩	+

【0025】

クリステンゼン培地での利用性 (アルカリ産生)	-
クエン酸塩	+
リンゴ酸塩	+
マレイン酸塩	+
マロン酸塩	+
プロピオン酸塩	+
グルコン酸塩	+

【0026】

コハク酸塩	+
グルコースよりガスの産生	-
糖より酸の産生 (窒素源にNH ₄ , H ₂ , PO ₄)	-
アドニトール	-
L (+)-アラビノース	-
セロビオース	-

9

ズルシトール
メリーエリスリトール
フラクトース

10

—
—
+

【0027】

D-ガラクトース
D-グルコース
グリセリン
イノシトール
イヌリン
ラクトース
マルトース
マンニトール

—
+
+
—
—
—
—
—
—

【0028】

マンノース
メレジトース
メリビオース
ラフィノース
L (+) -ラムノース
D-リボース
サリシン
L-ソルボース
ソルビトール

—
—
—
—
—
—
—
—
—

【0029】

スターチ
サッカロース
トレハロース
D-キシロース

—
+
(+)
+

【0030】6. その他の分析（化学分析等）

本菌株TM-1の主性状

グラム陽性菌の細菌で短時間培養菌では桿状を示し、定期的の細胞は球状～短桿状になる。また、V、Y字形の細胞も観られる。運動性なし、カタラーゼ非産生、グルコースを酸化的に分解し、酸を产生する。

【0031】本菌株TM-1の同定

グラム陽性の細菌で桿状細胞から球状細胞に変化し、V、Y字形（擬分歧）等の配列を示す菌属は、Coryneform群のArthrobacter属がある。本菌株の主性状から判断しArthrobacter属に属するものと判断した（他のCoryneform群も検索したが該当する菌属の記載はなかった）。

【0032】本菌株TM-1の諸性状とArthrobacter属の各菌種の諸性状を対比した結果A. simplexが糖よりの酸産生パターンは似ているが、澱粉の分解能、カタラーゼ産生能及びリトモスミルク培地での生育の特徴が一致しなかった。よって、本菌株をアースロバクター・エスピードTM-1 (Arthrobacter sp. TM-1)と同定命名した。本菌、アースロバクター・エスピード (Arthrobacter sp.) TM-1株は工業技術院微生物工業技術研究所に寄託した（微工研条寄第3676号、FER

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M BP-3676）。

【0033】本発明の有効成物AC8007物質を得るには、まず、上記の微生物またはAC8007物質を採取し得る量でAC8007物質を生産し得る能力を有する変異株又は変種を常法にしたがって培地内で好気的に培養する。本発明で例示する培地としては、上記アースロバクター属に属するAC8007物質生産菌を、イオン交換純水1Lあたり、イソプロピルアルコール10mL、酵母エキス0.3g、ペプトン3.0g、硝酸アンモニウム3.0g、リン酸カリウム0.4g、リン酸二ナトリウム1.5g、硫酸マグネシウム0.5g、硫酸マンガン10mg、硫酸亜鉛10mg、硝酸銅50μg、三酸化モリブデン10μg、炭酸カルシウム5.0gを含有する殺菌した培地100mLを収容した500mL容三角フラスコに植菌して、30°Cで3日間振とう培養すればよい。この培養物を、上記同様の培地100mLを含む500mL容三角フラスコに、1mL植菌して、30°Cで5日間振とう培養すればよい。

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【0034】このようにして得られた培養物からAC8007物質を採取するには、AC8007物質が主に培養液中に存在するため、例えば培養物を濾過し、その濾液に非水溶性有機溶媒、たとえば酢酸エチル、ブタノール、酢酸ブチルなどを加え、酸性pHにて抽出し、次

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いでアルカリpHで水に転溶し、さらに酸性pHで溶媒抽出すればよい。これをさらに、シリカゲル、アルミナ、合成吸着剤などによるクロマトグラフィーに付して、分離生成するか、また、高速液体クロマトグラフィーなどを用いて分離取得することもできる。また、得られたAC 8007物質は、公知の方法によりナトリウム塩などのアルカリ金属塩、カルシウム塩、マグネシウム塩等のアルカリ上類金属塩、アンモニウム塩、公知の非毒性有機アミンとの塩などの塩とすることもできる。このようにして得られたAC 8007物質は、前記したような理学的性質を有する。本発明のAC 8007物質は、以下に示す作用を有する。

【0035】(1) 抗腫瘍作用

1) Sarcoma 180に対する光増感治療効果
一群5匹のICRマウス20g(雄)の背部一ヶ所にsarcoma 180 (1×10^6 cells/ml)を0.05ml皮内接種した。2日後にAC 8007物質、ヘマトポルフィリン(HpD; シグマ社製)をそれぞれ15mgを3mlの0.1mMトリス塩酸緩衝液(pH7. *

	腫瘍の大きさ(mm)	日数(日)	3	4	5	6	7	8
			3	4	5	6	7	8
対 照	非照射	20.1	26.0	35.6	48.3	56.3	52.9	
	照射	21.5	27.0	36.0	48.0	58.2	58.1	
AC 8007物質 50mg/kg	非照射	21.1	28.2	38.1	46.6	57.6	58.1	
	照射	21.3	10.6	14.6	17.3	23.9	23.7	
ヘマトポルフィ リン50mg/kg	非照射	21.0	26.6	37.1	45.6	53.0	53.0	
	照射	20.6	17.6	23.3	31.9	60.0	45.5	

【0038】2) Sarcoma 180に対する作用
上記1)と同様の方法で、ICRマウスを1群3匹使用した。AC 8007物質、ヘマトポルフィリンジハイドロクロライド(NO. H-1875、純度約75%、シグマ社製)、ヘマトポルフィリン(NO. H-5518、純度約50%、シグマ社製)を、それぞれ25mg/kg、12.5mg/kg、6.3mg/kgとなるよう5mg/2ml、2.5mg/2ml、1.25mg/2ml溶液を0.1M

*4)を加えた生理食塩水に溶解し、0.2mlをマウス腹腔内に投与した。

【0036】10分後にペントバルビタール麻酔を行い、さらに10分後にハロゲンランプ(JR 15V 150WB)を使用したルミナエースL-150S(林時計製)により光照射を10分間、腫瘍部位に熱が伝わらないように行つた。3日目から腫瘍の長径と短径を測定し、以下の式に基き計算した値を腫瘍の大きさとした。

$$\text{長い径 (mm)} \times \text{短径 (mm)}$$

10 腫瘍の大きさ=----- 2

結果

AC 8007物質は、50mg/kg腹腔内投与後、20分に光を照射することにより表1および図5に示すとおり著明なsarcoma-180の増殖を抑制した。

【0037】

【表1】

40 【0039】

【表2】

トリス塩酸緩衝液(pH7.4)を加えた生理食塩水で調製した。この混合溶液0.2mlをマウスの腹腔内に投与し、治療効果を検べた。結果は、表2および図6に示すとおりで、AC 8007物質は、対照薬として使用したヘマトポルフィリンおよびヘマトポルフィリン2・HC1よりも効果が優れていることが確認された。

投与量 (mg/kg)	日 数	腫瘍の大きさ (mm ²)				
		日 数				
		3	4	5	6	7
対照	0	18.7	22.7	34.1	36.7	
AC 800 7物質	25	7.8	10.5	14.5	17.6	
	12.5	11.3	14.4	21.0	23.7	
	6.3	19.3	20.9	30.9	34.4	
ヘマトポル フイリン 2HCl	25	12.0	12.8	15.3	24.2	25.1
	12.5		12.9	16.3	25.6	32.0
	6.3		17.4	24.6	35.9	35.8
ヘマトポル フイリン	25	9.7	12.5	20.8	20.0	
	12.5		16.1	18.0	28.4	28.9
	6.3		18.1	20.7	39.0	36.7

【0040】(2)腫瘍診断への応用

ICRマウスの背部一ヶ所にSarcoma-180 (1×10^6 cells/ml)を0.05ml皮内接種した。2日後にAC8007物質50mg/kg投与し、光ガイドを通じて照射すると、腫瘍は蛍光により局所決定ができる。

【0041】(3)抗腫瘍作用

1) B-16メラノーマに対する光増感治療効果

一群3匹のBDF₁マウス20g(雄)の背部一ヶ所にB-16メラノーマ(2×10^7 cells/ml)を0.05ml皮内接種した。7日後にAC8007物質、ヘマトポルフイリン(HpD;シグマ社製)をそれぞれ50mg/kg、25mg/kg、12.5mg/kgとなるように10mg/2ml、5mg/2ml、2.5mg/2ml溶液を0.1Mトリス塩酸緩衝液(pH7.4)を加えた生理食塩水に溶解し、0.2mlをマウス腹腔内に投与した。10分後にペントバルビタール麻酔を行い、さらに10分後に

ハロゲンランプ(JR15V150WB)を使用したルミナースL-150S(林時計製)により光照射を10分間、腫瘍部位に熱が伝わらないように行った。8日目から腫瘍の長径と短径を測定し、以下の式に基き計算した値を腫瘍の大きさとした。

$$\text{腫瘍の大きさ} = \frac{\text{長径 (mm)} \times \text{短径 (mm)}}{2}$$

【0042】結果

AC8007物質は、50mg/kg、25mg/kg腹腔内投与後、20分に光を照射することにより表3および図8に示すとおり著名なB-16メラノーマの増殖を抑制し、HpDの光照射による光増感治療は表3および図9に示した。

【0043】

【表3】

投与量 (mg/kg)	腫瘍の大きさ (mm ²)	日 数					
		7	8	9	10	11	12
		対照	0	11.9	15.9	22.6	27.04
AC 800 7物質	50	11.7	6.9	13.1	14.4	18.2	20.2
	25	11.5	10.9	14.2	19.8	23.1	30.9
	12.5	11.0	12.2	17.4	21.7	28.1	32.5
H p D	50	11.3	11.10	13.3	15.0	18.0	24.9
	25	12.8	11.3	14.6	21.0	25.4	28.5
	12.5	12.1	11.2	17.3	25.2	28.9	43.3

【0044】以上のSarcoma-180、B-16

メラノーマを用いた腫瘍形成マウスに対し、AC 800
7物質は PDT により治療効果が認められ、ヒト肺由来
悪性腫瘍 A 549 株、ヒト大腸由来悪性腫瘍 A Z 521
株、ヒトメラノーマ G 361 株やヒト子宮頸部由来 He
La 細胞などのヒト由来腫瘍に対しても有効であると認められる。

【0045】(3) 急性毒性

AC 800 7 物質をマウスに 400 mg/kg 腹腔内投与し
ても死亡例はみられなかつた。

(4) マウス急性光毒性実験

光増感剤は生体に取り込まれ、直射日光にあたると光過敏症を引き起す。症状は初めヒフの紅斑と痛痒で始まり、その後水腫が発生し、数日後腫脹はひくがヒフの壞死が観察される。重篤な場合、昏睡状態になり死亡する*

* 例もある。

1) 実験方法

ICR (♂、22~25g) マウス (1群3匹) に AC 800 7、H p D をそれぞれについて 100 mg/kg、50 mg/kg となるように、腹腔内投与を行つた。投与直後からマウスの上部からハロゲンランプ (ルミナエース、林時計 (株)、JCR 15V、150WB) により 2 時間 (25~28°C 温度条件に維持) 照射した。この時、32000 ルックスであった。その後普通飼育し、マウス体重と生死を観察した。

2) 実験結果

その結果を表4に示す。

30 【0046】

【表4】

群	例 数	死 亡 例		
		1日目	2日目	3日目
コントロール 照射	3	0	0	0
AC 800 7 100 mg/kg (ip) 照射	3	0	0	0
物質 50 mg/kg (ip) 照射	3	0	0	0
H p D 100 mg/kg (ip) 照射	3	3		
50 mg/kg (ip) 照射	3	1	0	0

【0047】上記の表4に示す通り、AC 800 7 物質 (100 mg/kg、50 mg/kg) 投与群では死亡例は認められなかつた。H p D 100 mg/kg 投与群では全例翌日までに死亡し、50 mg/kg 投与群では翌日 1/3 例の死亡例が認められた。体重変化については図10に示す通

りであり、AC 800 7 物質群は無投与照射コントロール群と同じ様に体重の増加が認められた。H p D 50 mg/kg 投与群で生き残り 2/3 例の体重変化は翌日、翌々日まで体重減少が認められ、72 時間後に回復した。以上のように急性光毒性において AC 800 7 物質は H p

いにいくらべ高い安全性を認めた。

【0048】以上に述べたように、AC8007物質を、0.1Mトリス塩酸緩衝液を含む生理食塩水(pH7.4)に溶解し、腹腔内投与、静脈内投与、経口投与等により、本物質を投与することにより、腫瘍部位に行き渡っている期間に、光、レーザー、超音波、X線などの照射を行うものである。その結果、悪性腫瘍細胞を壊死にいたらしめ、悪性腫瘍の増殖を抑制することができる。したがつて、本発明の有効物質AC8007物質の投与量としては、1日成人1人当たり1～10mg/kgであり、投与方法としては、無菌緩衝液(pH7.4付近)を加えた生理食塩水に溶解し、静脈内投与、局所投与あるいは経口投与により行う。

【0049】

【発明の効果】本発明は、AC8007物質の光増感・蛍光作用により悪性腫瘍の治療ならびに診断に有効である。

【0050】実施例 1

(1) アースロバクター・エスピー・MT-1 (FERM BP-3676) を、イオン交換純水1Lあたり、イソプロピルアルコール10ml、酵母エキス0.3g、ペプトン3.0g、硝酸アンモニウム3.0g、リン酸カリウム0.4g、リン酸二ナトリウム1.5g、硫酸マグネシウム0.5g、硫酸マンガン10mg、硫酸亜鉛10mg、硫酸銅50μg、二酸化モリブデン10μg、炭酸カルシウム5.0gを含有する殺菌した培地100mlを収容した500ml容三角フラスコに植菌して、30℃で3日間振とう培養した。この培養物を、上記同様の培地100mlを含む500ml容三角フラスコに、1ml植菌して、30℃で5日間振とう培養した。この培養物を500ml容フラスコ200本を合わせた後、遠心分離によって除菌して培養上清約19Lを得た。

【0051】(2) 上記(1)で得た培養上清液を酢酸でpHを2.0に調整後、酢酸エチル8Lで有効成分を抽出した。この抽出液に水4Lを加え、水層のpHをアンモニア水で9.0に調整した後、抽出操作を行つた。分液した水層を約500mlにまで減圧濃縮した。濃縮液を吸着樹脂(ダイヤイオンHP-20、三菱化成社製)400mlのカラムに通した。水3Lで洗浄後、水3Lおよび80%アセトン水3Lを用いる直線型濃度勾配により溶出を行つた。最初の2Lを捨て、その後、17gづつの分画を行うと、フラクションNo. 87～150に有効成分が溶出された。これらのフラクションを集めて減圧濃縮して暗赤色粉末を得た。

【0052】この粉末を予めブタノール-エタノール-クロロホルム-アンモニア水(4:5:2:3)の混合溶媒で充填したシリカゲル(メルク社製、Art 7734、350ml)のカラムにチャージし、上記と同一の混合溶媒で溶出した。最初の600mlを捨て、その後、17gづつの分画を行うと、フラクションNo. 11～4

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0に有効成分が溶出された。これらのフラクションを集めて減圧濃縮してAC8007物質の暗赤色粉末を得た。

【0053】(3) 上記(2)で得た暗赤色粉末を、メタノール50%酢酸アンモニウム水溶液(55:45)の混合溶媒2mlに溶解し、これをオクタデシルシリカゲル(山村化学社製、YMC-GEL-ODS、662ml)のカラムにチャージし、前記と同一混合溶媒で溶出した。これを20mlづつ分画を行うとフラクションNo. 42～55に有効成分が溶出された。これらのフラクションを集めて減圧下メタノール留去した。残渣を吸着樹脂(三菱化成社製、ダイヤイオンHP-20、100ml)のカラムに通した。水1Lで洗浄後、80%アセトン水で溶出した。溶出液を減圧濃縮し、残渣を酢酸エチルに溶解した。

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【0054】この溶液を10mMエチレンジアミンテトラアセテート水溶液(pH2)で洗浄した後、酢酸エチル層を減圧濃縮した。残渣にヘキサンを加え、析出した沈殿物をガラスフィルター上に集め、減圧乾燥して精製されたAC8007物質(遊離液)を暗赤色粉末として得た。収量159mg。

【0055】(4) 上記(3)でAC8007物質(遊離液)10mgを4Nアンモニア水1mlに溶解した後、凍結乾燥してAC8007物質のアンモニウム塩を得た。収量11mg。

【0056】実施例 2

一群3匹のICRマウス、20g、雄の背部にsarcoma-180 (1×10^6 cells/ml)を0.05ml皮内に接種し、2日後にAC8007物質を腹腔内に投与した。10分後にペントバルビタール麻酔を行い、更にその後、10分後にハロゲンランプ(JCR-15V 150WB)を使用したルミナエースL150s(林時計製)により光照射を10分間、腫瘍部位に熱が伝わらないように行つた。腫瘍の大きさを3日目から8日目にかけて測定し、表1および表2に示すようなAC8007物質の著名な腫瘍増殖抑制作用が認められた。

【0057】実施例 3

AC8007物質を無菌生理食塩水(pH7.5付近に調整)に5mg/mlとなるよう溶解した。これを0.22μmミリポアフィルターで無菌濾過し、注射剤とした。

【図面の簡単な説明】

【図1】溶媒としてメタノールを用いたときのAC8007物質の可視部吸収スペクトルである。

【図2】溶媒として0.1N HCl・メタノールを用いたときのAC8007物質の可視部吸収スペクトルである。

【図3】AC8007物質の赤外線吸収スペクトルである。

【図4】AC8007物質のプロトン核磁気共鳴スペクトルである。

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【図5】AC8007物質の光増感治療効果を示した曲線である。

【図6】AC8007物質の光増感治療効果を示した曲線である。

【図7】AC8007物質の蛍光スペクトルである。

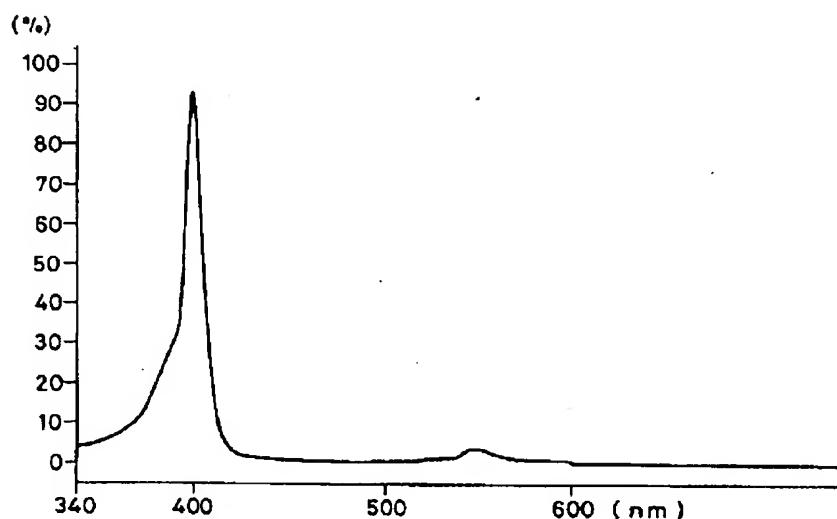
【図8】AC8007物質の光増感治療効果を示した曲線である。

*線である。

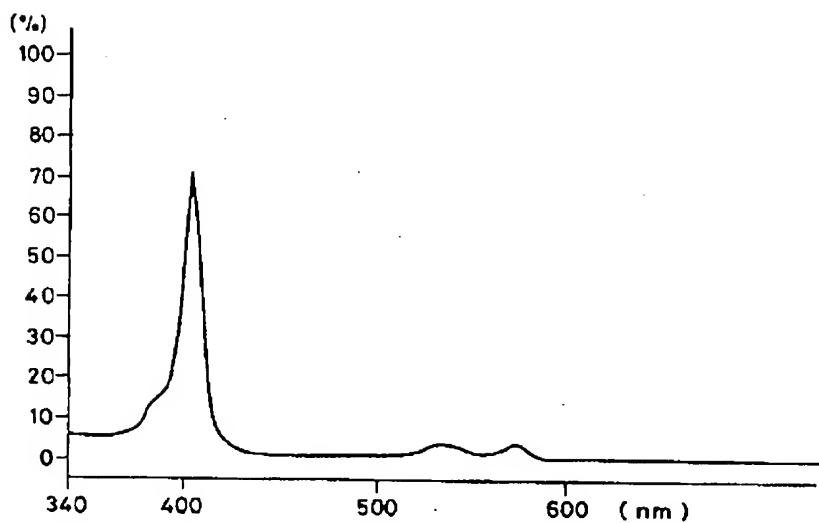
【図9】対照としてのヘマトポルフィリン(HpD)の光増感治療効果を示した曲線である。

【図10】AC8007物質およびヘマトポルフィリン(HpD)のマウス急性光毒性実験における体重変化の曲線である。

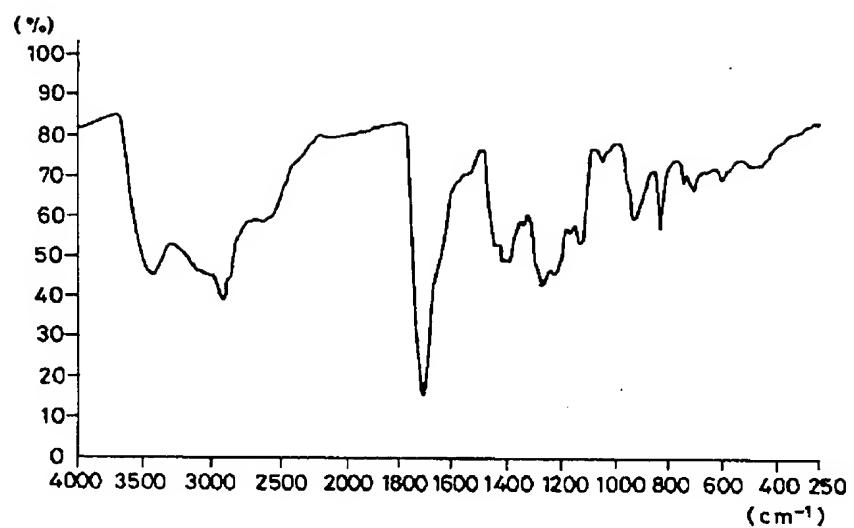
【図1】



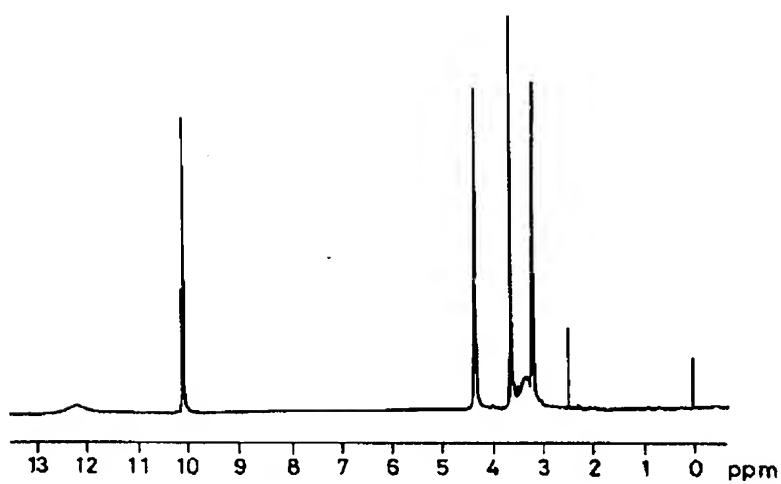
【図2】



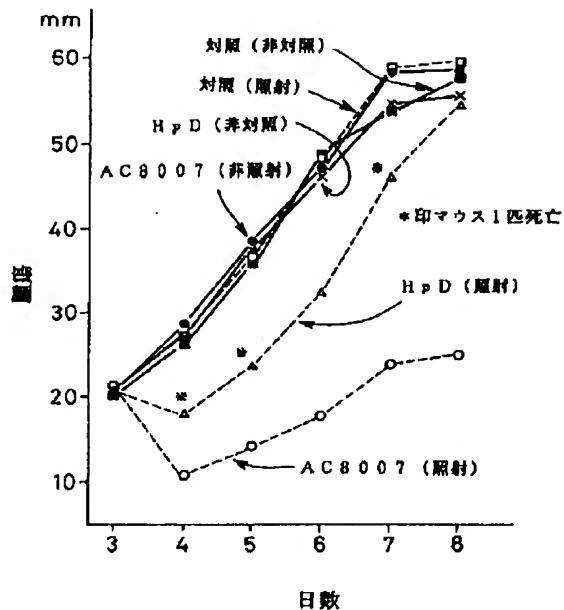
【図3】



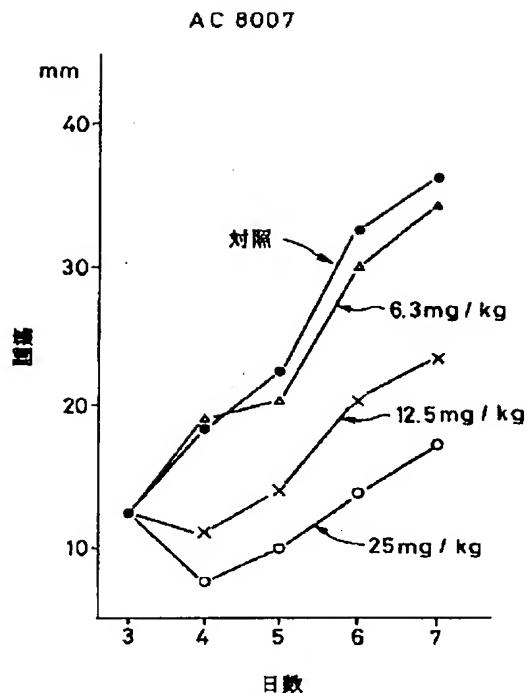
【図4】



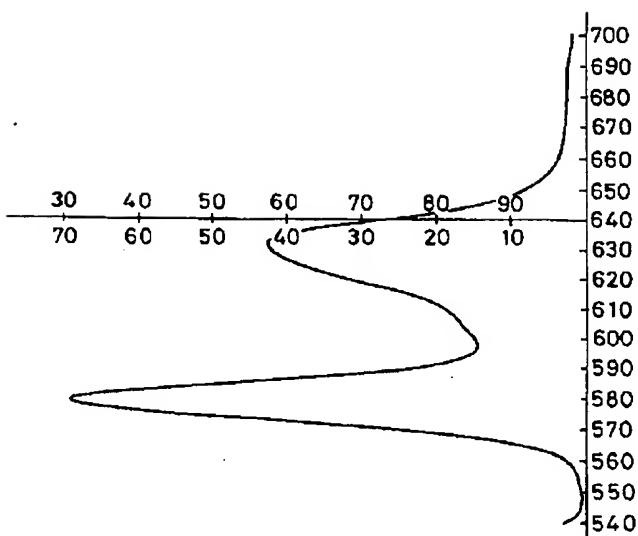
【図5】



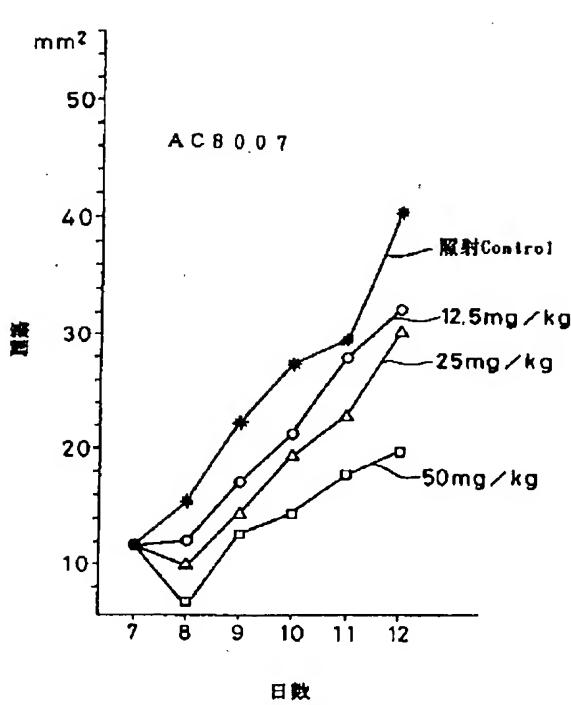
【図6】



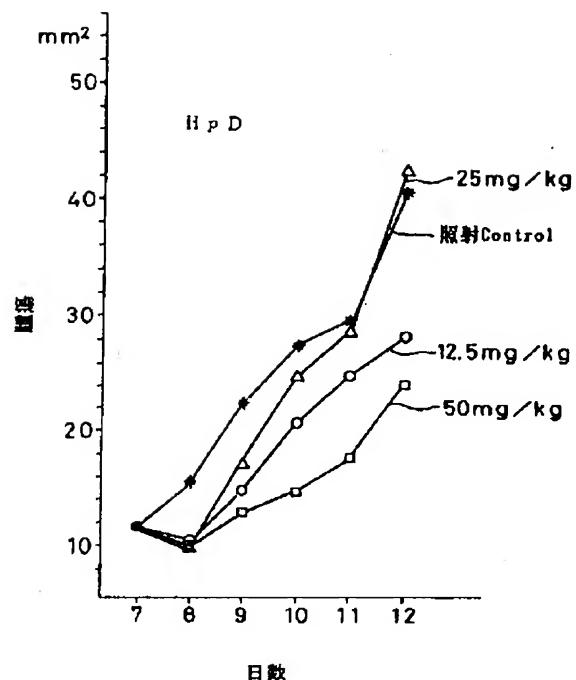
【図7】



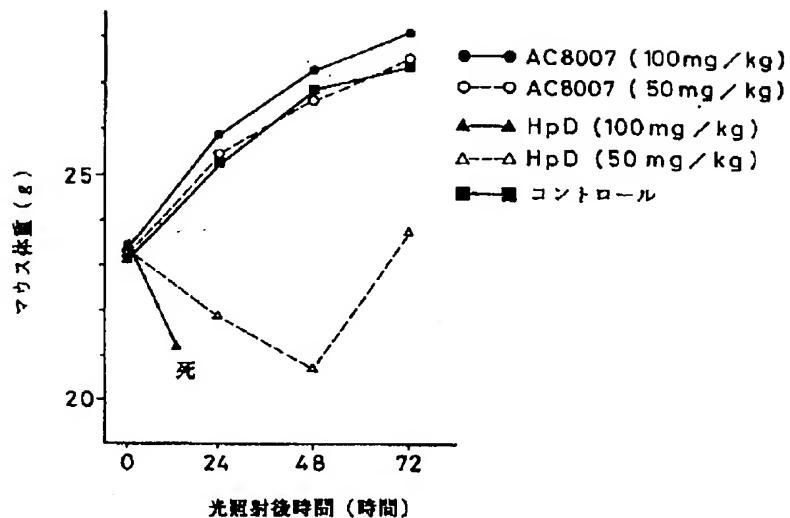
【図8】



【図9】



【図10】



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CLAIMS

[Claim(s)]

[Claim 1] A treating agent or a diagnostic agent of a malignant tumor making into an active principle AC8007 substance which has the following physicochemical properties, or its nontoxic salt.

(1) ultimate analysis value C: -- about 60% and H: -- about 5% and N: -- about 8% and Zn: about 8 to 10% mass-analysis [(2)] value 717 (based on MH^+ and FAB-MS)

(3) The molecular formula $C_{36}H_{36}O_8N_4Zn(4)$ visible-portion absorption spectrum [Formula 1]

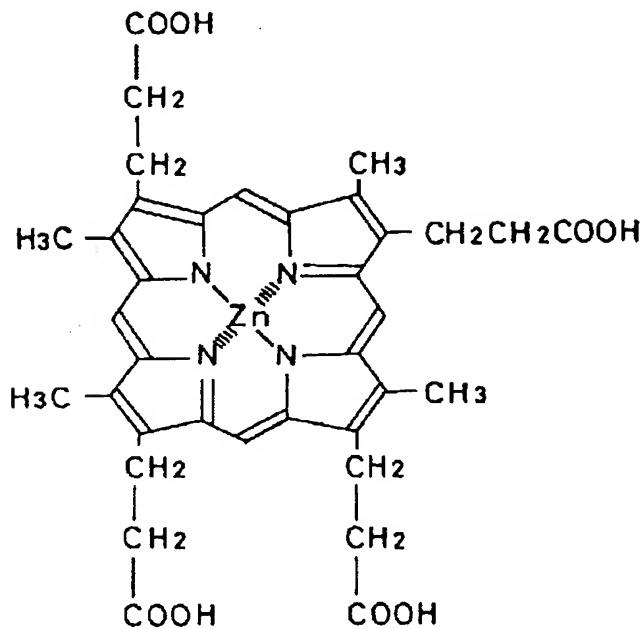
λ_{max} メタノール nm ($E_{1cm}^{1\%}$):

It has absorption characteristic 386 (shoulder) (750), 406 (3525), 538 (185), and near 574(190)nm at least. [Formula 2]

λ_{max} 0.1N HCl・メタノール nm ($E_{1cm}^{1\%}$):

(5) infrared absorption spectra which have absorption characteristic 386 (shoulder) (1275), 402 (4690), 560 (175), and near 591 (60) nm at least (the KBr method)

Soluble methanol to (6) solvents which have absorption characteristic 3420, 2920, 1705, 1400, 1275, 1130, 940, and near 835 cm^{-1} at least. To ethyl acetate, acetic acid, and dimethyl sulfoxide, fusibility, water, hexane, benzene -- insoluble (7) color-reaction potassium permanganate reaction and an iodine reaction -- a positivity, a ferric chloride method, the Dragendorff reaction, and a ninhydrin reaction -- negative (8) basicity and color dark red of an acid and neutral distinction acid (9) substance [Claim 2] A treating agent or a diagnostic agent of the malignant tumor according to claim 1, wherein AC8007 substance has the following presumed chemical constitution formula. [Formula 3]



[Claim 3]A manufacturing method of AC8007 substance cultivating AC8007 matter-production bacillus belonging to the Arthrobacter group to a culture medium, and extracting AC8007 substance from a culture subsequently.

[Claim 4]The manufacturing method according to claim 3 whose AC8007 matter-production bacilli belonging to the Arthrobacter group are Arthrobacter Espy and TM-1 (FERM BP-3676).

[Translation done.]

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- 2.**** shows the word which can not be translated.
3. In the drawings, any words are not translated.

DETAILED DESCRIPTION**[Detailed Description of the Invention]****[0001]**

[Industrial Application] This invention relates to the manufacturing method of the treating agent of a malignant tumor or a diagnostic agent, and AC8007 substance which makes an active principle AC8007 substance or its nontoxic salt.

[0002]

[Description of the Prior Art] The photochemical therapy (Photodynamic therapy:PDT) to a malignant tumor is developed, tens of years pass, many effective examples are checked, and it is used as the radical cure therapy or diagnostic agent of an early malignant tumor. The typical photosensitizer used for this PDT is a porphyrin derivative.

[0003] However, as for a porphyrin derivative, the following faults are **. (1) It is raised that the amount yield of (5) photochemical reactions with slow elimination from (4) normal cells which does not have absorption in the good long wave field of (2) tissue penetration which is not single chemically and which is not held for a long time at (3) tumor tissues is low etc.

[0004]

[Problem(s) to be Solved by the Invention] It is expected that development of the photosensitizer which can solve at least the one above problem is effective in future PDT. And they are ** ***** because of a malignant tumor therapy of a new and useful photosensitizer with few side effects. The purpose of this invention is to provide the manufacturing method of the treating agent of a malignant tumor or a diagnostic agent, and AC8007 substance which makes an active principle AC8007 substance or its nontoxic salt.

[0005]

[Means for Solving the Problem] All photosensitizers in old PDT are the substances compounded chemically, and a problem is ***** in a field of side effects. From inside of a natural physiological active substance which a microorganism produces, this invention persons extract a substance identified AC8007 substance (refer to JP,2-234688,A) from culture filtrate of Arthrobacter Espy and TM-1 (FERM BP No. 3676). This substance found out that it was a substance which has zinc. This invention persons found out that this substance was useful as a diagnostic agent as a treating agent which has the character as a feeling agent of light of PDT of a malignant tumor, found out a good manufacturing method of AC8007 substance further, and completed this invention.

[0006] AC8007 substance which is an effective substance of this invention has a physicochemical property as shown below at least.

- (1) ultimate analysis C: -- about 60% and H: -- about 5% and N: -- about 8% and Zn:about 8 to 10%
- (2) mass analysis 717 (based on MH^+ and FAB-MS)
- (3) The molecular formula $C_{38}H_{36}O_8N_4Zn(4)$ visible-portion absorption spectrum : drawing 1 (neutrality condition), drawing 2 (acid conditions)

[0007]

[Formula 4]

メタノール
 λ_{max} nm (E 1%
 1cm):

It has absorption characteristic 386 (shoulder) (750), 406 (3525), 538 (185), and near 574(190)nm at least. [0008]

[Formula 5]

0.1N HCl・メタノール
 λ_{max} nm (E 1%
 1cm):

It has absorption characteristic 386 (shoulder) (1275), 402 (4690), 560 (175), and near 591 (60) nm at least.

[0009](5) infrared-absorption-spectrum (KBr method): -- drawing 3 -- even if small, it has absorption characteristic 3420, 2920, 1705, 1400, 1275, 1130, 940, and near 835 cm^{-1} .

(6) It is insolubility to fusibility, water, hexane, and benzene in soluble methanol, the ethyl acetate, acetic acid, and dimethyl sulfoxide to a solvent. [0010]A color reaction potassium permanganate reaction and an iodine reaction (7) A positivity, a ferric chloride method, the Dragendorff reaction and a ninhydrin reaction -- color dark red (10) $^1\text{H-NMR}$ (it measures in 400-MHz, 27 **, and $d_6\text{DMSO}$):drawing 4 of negative (8) basicity and an acid and neutral distinction acid (9) substance

[0011](11) $^{13}\text{C-NMR}$ (it measures in 100 MHz, 27 **, and DMSO): The signal shown below at least was accepted. 174.20 (s), 147.62 (s), 147.54 (s), 146.83 (s), 146.77 (s), 146.73 (s), 139.44 (s), 139.30 (s), 136.65 (s), 136.54 (s), 97.06 (d), 96.95 (d), 37.41 (t), 21.63 (t), 21.59 (t), 11.46 (q)

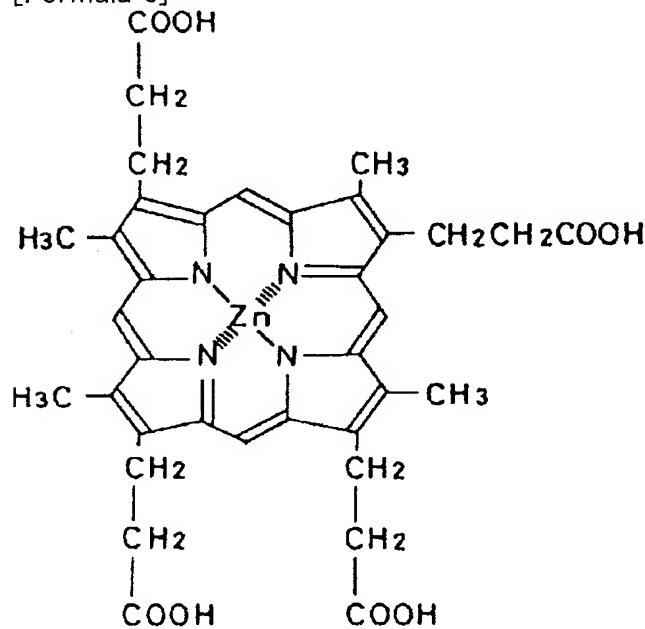
[0012](12) Thin layer chromatography (made in Tokyo Chemicals, spot film silica gel f use)
 $R_f=0.45$ [developing solvent: Chloroform methanol acetic acid (10:1.5:0.1)]

$R_f=0.37$ [developing solvent: Butanol-ethanol chloroform ammonia solution (4:5:2:4)]

[0013]It is presumed that it is a structure expressed with a following formula since AC8007 substance has the above character.

[0014]

[Formula 6]



[0015]In order to produce AC8007 substance which is an active principle of this invention, the fermenting method by culture of a microorganism is the most suitable. As a suitable microorganism for production, *Arthrobacter Espy TM-1* (*Arthrobacter* sp. TM-1: FERM BP No. 3676) can be mentioned. Bacteria is the bacteria TM-1 share separated from the soil of the Chinese cabbage field in Koka-cho, Koka-gun, Shiga-ken, is an example of the strain used the most effective in this invention, and when it shows the mycology character of a bacteria stock, it is as follows.

[0016]An identification test was carried out in identification of a bacteria stock according to "guidance of medicine bacteria identification, the 2nd edition, 1974", "three Microbiological Methods", etc. An experimental result was identified as contrasted with "guidance of medicine bacteria identification, the 2nd edition, 1974", "Bergey's Manual of Systematic Bacteriology Vol.1 (1984), Vol.2 (1986), Vol.3" (1989), etc. Culture temperature was performed at 28-30 **. (+: -- there is no statement in a positivity, a (+): weak positivity, -: negativity, NT: un-examining, and ND: literature -- NC: change of is not done)

[0017]The feature ordinary agar slant-medium circumference of experimental result 1. growth forms a round notch-like colony, and a center rises to convex. Soluble pigment is not produced, although it is translucent and humid and assumes off-white - light ochre.

Although ordinary agar flat-surface culture-medium growth is bad, it is grown to a line. Soluble pigment is not produced, although it is translucent and humid and assumes off-white - light ochre.
Liquid medium (peptone water)

It becomes muddy uniformly.

It becomes Ritzmos milk culture-medium alkali, and peptonizes.

[0018]2. GCmol %NT3. of DNA -- a long rod is shown in the first half of the feature culture of a main isoprenoid quinone NT4. gestalt, it curves and a size has some which show the shape of a V character by 0.8x4-5 micrometers. Bacteria which change in the second half of culture a 1.2x1.5-micrometer short rod - in the shape of a ball.

[0019]

5. Physiology and chemical description Gram's stain +KOH reaction - acid fast stain - capsule formation -[0020]

the OF test (Hugh-Leifson) NT OF test (it is $\text{NH}_4\text{H}_2\text{PO}_4$ to a source of N) -- growth [] by O aerobic -- growth [] by + aversion -- + growth temperature [] -- 42 ** - 37 *** 20 *** 10 ** NT

[0021]

Salt tolerance 0%+ 0.5%+ 3.0%NT 5.0% NT Growth pH 4.7 -5.6 + 9.0 + 10.0 -[0022]

Gelatin decomposition - amyloyisis - casein decomposition + esculin decomposition NT cellulose decomposition - tyrosine degradation NT Tween80 decomposition NT arginine decomposition NT catalase production - oxidase production NT[0023]

Lecithinase production - urease production (SSR). NT The urease production (Chris.) NT. Indore production - hydrogen sulfide production (lead.) acetate paper+ acetoin production (K_2HPO_4) -

acetoin production (NaCl) - MR test - nitrate reduction test (aerobic) - (detection of NO_2^-) -

(detection of NO_3^-)+[0024]

Availability (alkali production) citrate - malate + maleate in the Simmonds culture medium - Chestnut acid chloride - propionate - gluconate (+)

Succinate +[0025]

Availability (alkali production) in the Christensen culture medium Citrate + malate + maleate + chestnut acid chloride + propionate + gluconate +[0026]

It is production of gas from succinate + glucose. - It is production (it is $\text{NH}_4\text{H}_2\text{PO}_4$ to a nitrogen source) of acid from sugar. Adonitol - L(+)-arabinose - cellobiose-dulcitol - ****- erythritol - Fructose +[0027]

D-galactose - D-glucose + glycerin + inositol - inulin - lactose - malt sugar - Mannitol -[0028]

Mannose - MEREJITOSU - melibiose - Raffinose - L(+)-rhamnose - D-ribose - salicin - L-sorbose - sorbitol -[0029]

Starch - saccharose + Trehalose (+)

D-xylose +[0030]6. Other analysis (chemical analysis etc.)

Bacteria of the main description gram positive bacteria of bacteria stock TM-1 show a rod in short-time culture, and a cell of a stationary phase becomes a spherical - short rod. A cell of V and a Y shape is also seen. Motile nothing one, catalase un-producing, and glucose are decomposed oxidatively, and acid is produced.

[0031]A fungus in which it changes from a rod-like cell to a capitulum with bacteria of an identification Gram positive of bacteria stock TM-1, and arrangement of V, a Y shape (pseudo-branching), etc. is shown has an Arthrobacter group of a Coryneform group. It was judged that it belonged to an Arthrobacter group, judging from the main description of a bacteria stock (there was no statement of a fungus applicable although other Coryneform groups were searched).

[0032]As a result of contrasting many descriptions of bacteria stock TM-1, and many descriptions of each strain of an Arthrobacter group, A.simplex was [acid production pattern from sugar] alike, but the feature of growth by resolution, catalase production ability, and a Ritmos milk culture medium of starch was not in agreement. Therefore, identification naming of the bacteria stock was carried out with Arthrobacter Espy TM-1 (Arthrobacter sp.TM-1). Bacteria and Arthrobacter Espy (Arthrobactersp.) TM-1 share were deposited with the Fermentation Research Institute, the Agency of Industrial Science and Technology (FERM BP No. 3676, FERM BP-3676).

[0033]In order to obtain effective product AC8007 substance of this invention, a variant or a variety which has the ability to produce AC8007 substance is first cultivated aerobically in a culture medium in accordance with a conventional method in quantity which can extract the above-mentioned microorganism or AC8007 substance. As a culture medium illustrated by this invention, AC8007 matter-production bacillus belonging to the above-mentioned Arthrobacter group, Per [ionic exchange pure water 1l], 10 ml of isopropyl alcohol, the yeast extract 0.3g, The peptone 3.0g, the ammonium nitrate 3.0g, the potassium phosphate 0.4g, 1.5 g of phosphoric acid disodium, the magnesium sulfate 0.5g, 10 mg of manganese sulfate, What is necessary is to carry out inoculation to a 500ml Erlenmeyer flask which accommodated 100 ml of sterilized culture media containing 10 mg of sulfate of zinc, cupric nitrate 50mug, molybdenum-trioxide 10mug, and the calcium carbonate 5.0g, and just to carry out shaking culture for three days at 30 **. What is necessary is to carry out 1-ml inoculation of this culture to a 500ml Erlenmeyer flask containing 100 ml of the same culture media as the above, and just to carry out shaking culture to it for five days at 30 **.

[0034]Thus, in order to extract AC8007 substance from an obtained culture, What is necessary is to filter a culture, for example, to add a nonaqueous solubility organic solvent, for example, ethyl acetate, butanol, butyl acetate, etc. to the filtrate, to extract by acid pH, to **** by alkali pH subsequently to water, and just to carry out solvent extraction by acid pH further, since AC8007 substance mainly exists in culture filtrate. This is further given to chromatography by silica gel, alumina, a synthetic adsorbent material, etc., separation generation can be carried out or separation acquisition can also be carried out using high performance liquid chromatography etc. AC8007 obtained substance can also be made into salts, such as alkaline earth metal salt, such as alkali metal salt, such as sodium salt, calcium salt, and magnesium salt, ammonium salt, and a salt with publicly known nontoxic organic amine, by a publicly known method. Thus, AC8007 obtained substance has the physicochemical property which was described above. AC8007 substance of this invention has the operation shown below.

[0035](1) The 0.05-ml intracutaneous vaccination of sarcoma-180 (1×10^8 cells/ml) was carried out behind [one] the ICR mouse 20g (male) of five photosensitization curative effect groups to antitumor action 1Sarcoma-180. Two days afterward, AC8007 substance and hematoporphyrin (HpD; made by a sigma company) were dissolved in a physiological saline which added 3 ml of 0.1mM trichloride buffer solution (pH 7.4) for 15 mg, respectively, and it medicated mouse intraperitoneal

with 0.2 ml.

[0036]It is ***** so that heat may not get across an optical exposure to a tumor site for 10 minutes by lumina ace L-150S (product made from a wood clock) which performed pentobarbital anesthesia 10 minutes afterward, and also uses a halogen lamp (JR15V150WB) 10 minutes afterward. A major axis and a minor axis of a tumor were measured from the 3rd, and a value calculated based on the following formulas was made into a size of a tumor.

長い径 (mm) × 短径 (mm)

腫瘍の大きさ =

2

After 50 mg/kg intraperitoneal injection, result AC8007 substance controlled growth of prominent sarcoma-180 as by irradiating with light in 20 minutes showed it to Table 1 and drawing 5.

[0037]

[Table 1]

	腫瘍の大きさ (mm)						
	日数 (日)	3	4	5	6	7	8
対照	非照射	20.1	26.0	35.6	48.3	56.3	52.9
	照射	21.5	27.0	36.0	48.0	58.2	58.1
AC8007物質 50mg/kg	非照射	21.1	28.2	38.1	46.6	57.6	58.1
	照射	21.3	10.6	14.6	17.3	23.9	23.7
ヘマトポルフィ リン50mg/kg	非照射	21.0	26.6	37.1	45.6	53.0	53.0
	照射	20.6	17.6	23.3	31.9	60.0	45.5

[0038]2) By the same method as the operation above 1 to Sarcoma-180, one groups [three] used an ICR mouse. AC8007 substance, hematoporphyrin dihydrochloride (NO.H-1875 and about 75% of purity) Sigma company make, hematoporphyrin (NO.H-5518 and about 50% of purity) 5 mg/2 ml, 2.5 mg/2 ml, and 1.25 mg/2ml solution were prepared with a physiological saline which added 0.1M trischloride buffer solution (pH 7.4) so that it might become 25 mg/kg, 12.5 mg/kg, and 6.3 mg/kg about sigma company make, respectively. Intraperitoneal [of a mouse] is medicated with 0.2 ml of this mixed solution, and it is a curative effect ** poor ** A result is as being shown in Table 2 and drawing 6, and it was checked that AC8007 substance excels in an effect hematoporphyrin and hematoporphyrin 2 and HCl used as a control drug.

[0039]

[Table 2]

	投与量	腫瘍の大きさ (mm ²)				
		日 数				
	(mg/kg)	3	4	5	6	7
対照	0	18.7	22.7	34.1	36.7	
AC8007物質	25	7.8	10.5	14.5	17.6	
	12.5	11.3	14.4	21.0	23.7	
	6.3	19.3	20.9	30.9	34.4	
ヘマトポル フイリン 2HCl	25	12.0	12.8	15.3	24.2	25.1
	12.5		12.9	16.3	25.6	32.0
	6.3		17.4	24.6	35.9	35.8
ヘマトポル フイリン	25	9.7	12.5	20.8	20.0	
	12.5		16.1	18.0	28.4	28.9
	6.3		18.1	20.7	39.0	36.7

[0040](2) The 0.05-ml intracutaneous vaccination of Sarcoma-180 (1×10^8 cells/ml) was carried out behind [one] the application ICR mouse to tumor diagnosis. If AC8007 substance 50 mg/kg administration is carried out and it glares through lightguide in two days, the tumor can perform partial determination according to fluorescence.

[0041](3) The 0.05-ml intracutaneous vaccination of the B-16 melanoma (2×10^7 cells/ml) was carried out behind [one] the BDF₁ mouse 20g (male) of three photosensitization curative effect groups to B-antitumor action 1 16 melanoma. AC8007 substance and hematoporphyrin (HpD; made by a sigma company) in seven days, respectively 50 mg/kg, 10 mg/2 ml, 5 mg/2 ml, and 2.5 mg/2ml solution were dissolved in the physiological saline which added 0.1M trichloride buffer solution (pH 7.4) so that it might become 25mg [kg] /and 12.5 mg/kg, and it medicated mouse intraperitoneal with 0.2 ml. Pentobarbital anesthesia was performed 10 minutes afterward, and also it carried out so that heat might not get across an optical exposure to a tumor site for 10 minutes by lumina ace L-150S (product made from a wood clock) which uses a halogen lamp (JR15V150WB) 10 minutes afterward. The major axis and minor axis of a tumor were measured from the 8th, and the value calculated based on the following formulas was made into the size of a tumor.

長径 (mm) × 短径 (mm)

腫瘍の大きさ =

2

[0042]After 50 mg/kg and 25 mg/kg intraperitoneal injection, result AC8007 substance controlled growth of B-16 prominent melanoma as by irradiating with light in 20 minutes showed it to Table 3 and drawing 8, and the photosensitization therapy by the optical exposure of HpD was shown in Table 3 and drawing 9.

[0043]

[Table 3]

投与量 (mg/kg)	腫瘍の大きさ (mm ²)	日 数					
		7	8	9	10	11	12
	対照	0	11.9	15.9	22.6	27.04	27.46
AC8007 物質	50	11.7	6.9	13.1	14.4	18.2	20.2
	25	11.5	10.9	14.2	19.8	23.1	30.9
	12.5	11.0	12.2	17.4	21.7	28.1	32.5
HpD	50	11.3	11.10	13.3	15.0	18.0	24.9
	25	12.8	11.3	14.6	21.0	25.4	28.5
	12.5	12.1	11.2	17.3	25.2	28.9	43.3

[0044]As opposed to the tumorigenesis mouse using Sarcoma-180 of a more than, and B-16 melanoma, A curative effect is accepted by PDT and AC8007 substance is accepted to be effective also to the Homo sapiens origin tumors, such as Homo sapiens lung origin malignant tumor A549 share, 521 shares of Homo sapiens large intestine origin malignant tumors AZ, Homo sapiens melanoma G361 share, a Homo sapiens uterine cervix origin Hela cell.

[0045](3) Even if it carried out 400 mg/kg intraperitoneal injection of the acute toxicity AC8007 substance at the mouse, the example of death was not seen.

(4) A mouse acuteness phototoxic experiment photosensitizer is incorporated into a living body, and if it hits direct sunlight, it will cause photosensitivity. Condition starts in the erythema and pain and itch of HIFU at first, an edema occurs after that, and a necrosis of HIFU is observed although the swelling after several days pulls. When critical, there is also an example which will be in a coma and dies.

1) It injected intraperitoneally so that it might become an experimental method ICR (**, 22-25g) mouse (one groups [three]) with 100 mg/kg and 50 mg/kg about each about AC8007 and HpD. It glared from the upper part of immediately after administration to the mouse for 2 hours (it maintains to 25-28 ** temperature conditions) with the halogen lamp (the lumina ace, Wood Clock, JCR15V, 150WB). At this time, it was 32000 luxs. It usually bred after that and mouse weight and life and death were observed.

2) an experiment result -- the result is shown in Table 4.

[0046]

[Table 4]

群	例 数	死 亡 例		
		1日目	2日目	3日目
コントロール 照射	3	0	0	0
AC8007 100 mg/kg (ip) 照射 物質 50 mg/kg (ip) 照射	3	0	0	0
HpD 100 mg/kg (ip) 照射 50 mg/kg (ip) 照射	3	3	1	0
	3			0

[0047]By AC8007 substance (100 mg/kg, 50 mg/kg) administration group, the example of death was not accepted as shown in the above-mentioned table 4. It died from the HpD100 mg/kg administration group by all the days following an example, and 1/3 example of death was accepted by 50 mg/kg administration group on the next day. It is as a weight change being shown in drawing 10, and, as for AC8007 substance group, the increase in weight was accepted like the unprescribed a medicine for the patient exposure control group. It survived by the HpD50 mg/kg administration group, and the loss weight was accepted to following **** and the weight change of 2/3 example was recovered 72 hours afterward on the next day. In acute phototoxicity, AC8007 substance accepted high safety compared with HpD as mentioned above.

[0048]As stated above, dissolve AC8007 substance in the included physiological saline (pH 7.4), and 0.1M trichloride buffer solution by intraperitoneal injection, intravenous administration, internal use, etc. By prescribing this substance for the patient, it goes to a tumor site and a ***** period is irradiated with light, laser, an ultrasonic wave, X-rays, etc. As a result, it can close, if a malignant tumor cell is in a necrosis, and growth of a malignant tumor can be controlled. Therefore, as a dose of effective substance AC8007 substance of this invention, it is 1 per one-day adult - 10 mg/kg, and as a medication method, it dissolves in the physiological saline which added sterile buffer solution (pH 7.4 neighborhood), and intravenous administration, local administration, or internal use performs.

[0049]

[Effect of the Invention]This invention is effective in the therapy and diagnosis of a malignant tumor by the photosensitization and scintillation effect of AC8007 substance.

[0050]Example 1(1) Arthrobacter Espy and MT-1 (FERM BP-3676), Per [ionic exchange pure water 1l], 10 ml of isopropyl alcohol, the yeast extract 0.3g, The peptone 3.0g, the ammonium nitrate 3.0g, the potassium phosphate 0.4g, 1.5 g of phosphoric acid disodium, the magnesium sulfate 0.5g, 10 mg of manganese sulfate, Inoculation was carried out to the 500ml Erlenmeyer flask which accommodated 100 ml of sterilized culture media containing 10 mg of sulfate of zinc, copper sulfate 50mug, molybdenum-trioxide 10mug, and the calcium carbonate 5.0g, and shaking culture was carried out for three days at 30 **. 1-ml inoculation of this culture was carried out to the 500ml Erlenmeyer flask containing 100 ml of the same culture media as the above, and shaking culture was carried out to it for five days at 30 **. This culture was disinfected by centrifugal separation, after setting 200 500-ml ** flasks, and about 19 l. of culture supernatants were obtained.

[0051](2) Acetic acid extracted the culture supernatant fluid obtained above (1), and the ethyl acetate 8l extracted the active principle after adjusting pH to 2.0. It is ***** about the extract operation after it adds the water 4l to this extract and an ammonia solution adjusts pH of a water layer to 9.0. Vacuum concentration of the separated water layer was carried out even to about 500

ml. It let the concentrate pass in the column of 400 ml of adsorption resin (diagram ion HP-20, Mitsubishi Kasei Corp. make). It is ***** about elution with the water 3l by the linear-model concentration gradient using after washing, the water 3l, and the 80% acetone water 3l. When 2 l. of the beginning was thrown away and every 17g of fractionation was performed after that, fraction No.87 – 150 were eluted in the active principle. Vacuum concentration of these fractions was collected and carried out, and dark red powder was obtained.

[0052]This powder was charged in the column of the silica gel (the Merck Co. make, Art7734, 350 ml) beforehand filled up with the mixed solvent of the butanol-ethanol chloroform ammonia solution (4:5:2:3), and it was eluted with the same mixed solvent as the above. When 600 ml of the beginning was thrown away and every 17g of fractionation was performed after that, fraction No.11 – 40 were eluted in the active principle. Vacuum concentration of these fractions was collected and carried out, and the dark red powder of AC8007 substance was obtained.

[0053](3) The dark red powder obtained above (2) was dissolved in 2 ml of mixed solvents of methanol 50% ammonium acetate solution (55:45), this was charged in the column of octadecyl silica gel (mountain village chemicals company make, YMC-GEL-ODS, 662 ml), and it was eluted with the same mixed solvent as the above. When it performed 20 ml of fractionation at a time, fraction No.42 – 55 were eluted [this] in the active principle. These fractions were collected and bottom methanol distilling off of decompression was carried out. It let residue pass in the column of adsorption resin (the Mitsubishi Kasei Corp. make, diagram ion HP-20, 100 ml). It was eluted with acetone water 80% after washing with the water 1l. Vacuum concentration of the eluate was carried out and residue was dissolved in ethyl acetate.

[0054]After 10mM ethylenediamine tetra acetate solution (pH 2) washed this solution, vacuum concentration of the ethyl acetate layer was carried out. AC8007 substance (isolation liquid) refined by collecting the settling which added hexane to residue and deposited on a glass filter, and carrying out reduced pressure drying was obtained as dark red powder. The yield of 159 mg.

[0055](4) After dissolving 10 mg of AC8007 substances (isolation liquid) in 1 ml of 4N ammonia solutions above (3), it freeze-dried and the ammonium salt of AC8007 substance was obtained. The yield of 11 mg.

[0056]sarcoma-180 (1×10^8 cells/ml) was inoculated behind the ICR mouse of three example 2 groups, 20 g, and a male in a 0.05-ml hide, and it medicated intraperitoneal with AC8007 substance two days afterward. It is ***** so that pentobarbital anesthesia may be performed in 10 minutes and also heat may not get across an optical exposure to a tumor site for 10 minutes after that by the lumina ace L150s (product made from a wood clock) who uses a halogen lamp (JCR15V150WB) 10 minutes afterward. The size of a tumor was measured from the 3rd day to the 8th day, and the prominent tumor growth depressant action of AC8007 substance as shown in Table 1 and 2 was accepted.

[0057]Example 3AC8007 substance was dissolved so that it might become a sterile physiological saline (it adjusts to the pH 7.5 neighborhood) in ml and 5mg /. Sterile filtration of this was carried out with a 0.22-micrometer millipore filter, and it was considered as injections.

[Translation done.]

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TECHNICAL FIELD

[Industrial Application] This invention relates to the manufacturing method of the treating agent of a malignant tumor or a diagnostic agent, and AC8007 substance which makes an active principle AC8007 substance or its nontoxic salt.

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PRIOR ART

[Description of the Prior Art] The photochemical therapy (Photodynamic therapy:PDT) to a malignant tumor is developed, tens of years pass, many effective examples are checked, and it is used as the radical cure therapy or diagnostic agent of an early malignant tumor. The typical photosensitizer used for this PDT is a porphyrin derivative.

[0003] However, as for a porphyrin derivative, the following faults are **. (1) It is raised that the amount yield of (5) photochemical reactions with slow elimination from (4) normal cells which does not have absorption in the good long wave field of (2) tissue penetration which is not single chemically and which is not held for a long time at (3) tumor tissues is low etc.

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EFFECT OF THE INVENTION

[Effect of the Invention] This invention is effective in the therapy and diagnosis of a malignant tumor by the photosensitization and scintillation effect of AC8007 substance.

[0050] Example 1(1) Arthrobacter Espy and MT-1 (FERM BP-3676), Per [ionic exchange pure water 1l], 10 ml of isopropyl alcohol, the yeast extract 0.3g, The peptone 3.0g, the ammonium nitrate 3.0g, the potassium phosphate 0.4g, 1.5 g of phosphoric acid disodium, the magnesium sulfate 0.5g, 10 mg of manganese sulfate, Inoculation was carried out to the 500ml Erlenmeyer flask which accommodated 100 ml of sterilized culture media containing 10 mg of sulfate of zinc, copper sulfate 50mug, molybdenum-trioxide 10mug, and the calcium carbonate 5.0g, and shaking culture was carried out for three days at 30 **. 1-ml inoculation of this culture was carried out to the 500ml Erlenmeyer flask containing 100 ml of the same culture media as the above, and shaking culture was carried out to it for five days at 30 **. This culture was disinfected by centrifugal separation, after setting 200 500-ml ** flasks, and about 19 l. of culture supernatants were obtained.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] It is expected that development of the photosensitizer which can solve at least the one above problem is effective in future PDT. And they are ** ***** because of a malignant tumor therapy of a new and useful photosensitizer with few side effects. The purpose of this invention is to provide the manufacturing method of the treating agent of a malignant tumor or a diagnostic agent, and AC8007 substance which makes an active principle AC8007 substance or its nontoxic salt.

[Translation done.]

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MEANS

[Means for Solving the Problem] All photosensitizers in old PDT are the substances compounded chemically, and a problem is ***** in a field of side effects. From inside of a natural physiological active substance which a microorganism produces, this invention persons extract a substance identified AC8007 substance (refer to JP,2-234688,A) from culture filtrate of Arthrobacter Espy and TM-1 (FERM BP No. 3676). This substance found out that it was a substance which has zinc. This invention persons found out that this substance was useful as a diagnostic agent as a treating agent which has the character as a feeling agent of light of PDT of a malignant tumor, found out a good manufacturing method of AC8007 substance further, and completed this invention.

[0006] AC8007 substance which is an effective substance of this invention has a physicochemical property as shown below at least.

(1) ultimate analysis C: -- about 60% and H: -- about 5% and N: -- about 8% and Zn: about 8 to 10%

(2) mass analysis 717 (based on MH^+ and FAB-MS)

(3) The molecular formula $C_{38}H_{36}O_8N_4Zn(4)$ visible-portion absorption spectrum : drawing 1

(neutrality condition), drawing 2 (acid conditions)

[0007]

[Formula 4]

λ_{max} メタノール nm ($E \frac{1\%}{1cm}$):

It has absorption characteristic 386 (shoulder) (750), 406 (3525), 538 (185), and near 574(190)nm at least. [0008]

[Formula 5]

λ_{max} 0.1N HCl・メタノール nm ($E \frac{1\%}{1cm}$):

It has absorption characteristic 386 (shoulder) (1275), 402 (4690), 560 (175), and near 591 (60) nm at least.

[0009](5) infrared-absorption-spectrum (KBr method): -- drawing 3 -- even if small, it has

absorption characteristic 3420, 2920, 1705, 1400, 1275, 1130, 940, and near 835 cm^{-1} .

(6) It is insolubility to fusibility, water, hexane, and benzene in soluble methanol, the ethyl acetate, acetic acid, and dimethyl sulfoxide to a solvent. [0010]A color reaction potassium permanganate reaction and an iodine reaction (7) A positivity, a ferric chloride method, the Dragendorff reaction

and a ninhydrin reaction -- color dark red (10) 1H -NMR(it measures in 400-MHz, 27 **, and d_6 DMSO):drawing 4 of negative (8) basicity and an acid and neutral distinction acid (9) substance

[0011](11) ^{13}C -NMR (it measures in 100 MHz, 27 **, and DMSO): The signal shown below at least

was accepted. 174.20 (s), 147.62 (s), 147.54 (s), 146.83 (s), 146.77 (s), 146.73 (s), 139.44 (s), 139.30 (s), 136.65 (s), 136.54 (s), 97.06 (d), 96.95 (d), 37.41 (t), 21.63 (t), 21.59 (t), 11.46 (q)

[0012](12) Thin layer chromatography (made in Tokyo Chemicals, spot film silica gel f use)

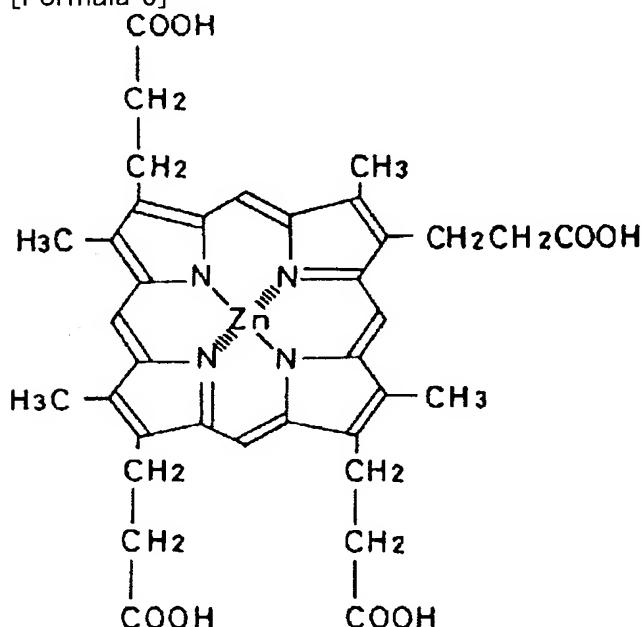
Rf=0.45[developing solvent: Chloroform methanol acetic acid (10:1.5:0.1)]

Rf=0.37[developing solvent: Butanol-ethanol chloroform ammonia solution (4:5:2:4)]

[0013]It is presumed that it is a structure expressed with a following formula since AC8007 substance has the above character.

[0014]

[Formula 6]



[0015]In order to produce AC8007 substance which is an active principle of this invention, the fermenting method by culture of a microorganism is the most suitable. As a suitable microorganism for production, Arthrobacter Espy TM-1 (Arthrobactersp.TM-1: FERM BP No. 3676) can be mentioned. Bacteria is the bacteria TM-1 share separated from the soil of the Chinese cabbage field in Koka-cho, Koka-gun, Shiga-ken, is an example of the strain used the most effective in this invention, and when it shows the mycology character of a bacteria stock, it is as follows.

[0016]An identification test was carried out in identification of a bacteria stock according to "guidance of medicine bacteria identification, the 2nd edition, 1974", "three Microbiological Methods", etc. An experimental result was identified as contrasted with "guidance of medicine bacteria identification, the 2nd edition, 1974", "Bergey's Manual of Systematic Bacteriology Vol.1 (1984), Vol.2 (1986), Vol.3" (1989), etc. Culture temperature was performed at 28-30 **. (+: -- there is no statement in a positivity, a (+):weak positivity, -:negativity, NT:un-examining, and ND:literature -- NC:change of is not done)

[0017]The feature ordinary agar slant-medium circumference of experimental result 1. growth forms a round notch-like colony, and a center rises to convex. Soluble pigment is not produced, although it is translucent and humid and assumes off-white - light ochre.

Although ordinary agar flat-surface culture-medium growth is bad, it is grown to a line. Soluble pigment is not produced, although it is translucent and humid and assumes off-white - light ochre. Liquid medium (peptone water)

It becomes muddy uniformly.

It becomes Ritmos milk culture-medium alkali, and peptonizes.

[0018]2. GCmol %NT3. of DNA -- a long rod is shown in the first half of the feature culture of a

main isoprenoid quinone NT4. gestalt, it curves and a size has some which show the shape of a V character by 0.8x4-5 micrometers. Bacteria which change in the second half of culture a 1.2x1.5-micrometer short rod - in the shape of a ball.

[0019]

5. Physiology and chemical description Gram's stain +KOH reaction - acid fast stain - capsule formation -[0020]

the OF test (Hugh-Leifson) NT OF test (it is $\text{NH}_4\text{H}_2\text{PO}_4$ to a source of N) -- growth [] by O aerobic -- growth [] by + aversion -- + growth temperature [] -- 42 ** - 37 *** 20 *** 10 ** NT [0021]

Salt tolerance 0%+ 0.5%+ 3.0%NT 5.0% NT Growth pH 4.7 -5.6 + 9.0 + 10.0 -[0022]

Gelatin decomposition - amylolysis - casein decomposition + esculin decomposition NT cellulose decomposition - tyrosine degradation NT Tween80 decomposition NT arginine decomposition NT catalase production - oxidase production NT[0023]

Lecithinase production - urease production (SSR). NT The urease production (Chris.) NT. Indore production - hydrogen sulfide production (lead.) acetate paper+ acetoin production (K_2HPO_4) - acetoin production (NaCl) - MR test - nitrate reduction test (aeriosis) - (detection of NO_2^-) - (detection of NO_3^-)+[0024]

Availability (alkali production) citrate - malate + maleate in the Simmonds culture medium - Chestnut acid chloride - propionate - gluconate (+)

Succinate +[0025]

Availability (alkali production) in the Christensen culture medium Citrate + malate + maleate + chestnut acid chloride + propionate + gluconate +[0026]

It is production of gas from succinate + glucose. - It is production (it is $\text{NH}_4\text{H}_2\text{PO}_4$ to a nitrogen source) of acid from sugar. Adonitol - L(+)-arabinose - cellobiose-dulcitol - ***- erythritol - Fructose +[0027]

D-galactose - D-glucose + glycerin + inositol - inulin - lactose - malt sugar - Mannitol -[0028]

Mannose - MEREJITOSU - melibiose - Raffinose - L(+)-rhamnose - D-ribose - salicin - L-sorbose - sorbitol -[0029]

Starch - saccharose + Trehalose (+)

D-xylose +[0030]6. Other analysis (chemical analysis etc.)

Bacteria of the main description gram positive bacteria of bacteria stock TM-1 show a rod in short-time culture, and a cell of a stationary phase becomes a spherical - short rod. A cell of V and a Y shape is also seen. Motile nothing one, catalase un-producing, and glucose are decomposed oxidatively, and acid is produced.

[0031]A fungus in which it changes from a rod-like cell to a capitulum with bacteria of an identification Gram positive of bacteria stock TM-1, and arrangement of V, a Y shape (pseudo-branching), etc. is shown has an Arthrobacter group of a Coryneform group. It was judged that it belonged to an Arthrobacter group, judging from the main description of a bacteria stock (there was no statement of a fungus applicable although other Coryneform groups were searched).

[0032]As a result of contrasting many descriptions of bacteria stock TM-1, and many descriptions of each strain of an Arthrobacter group, A.simplex was [acid production pattern from sugar] alike, but the feature of growth by resolution, catalase production ability, and a Ritmos milk culture medium of starch was not in agreement. Therefore, identification naming of the bacteria stock was carried out with Arthrobacter Espy TM-1 (Arthrobacter sp.TM-1). Bacteria and Arthrobacter Espy (Arthrobactersp.) TM-1 share were deposited with the Fermentation Research Institute, the Agency of Industrial Science and Technology (FERM BP No. 3676, FERM BP-3676).

[0033]In order to obtain effective product AC8007 substance of this invention, a variant or a variety which has the ability to produce AC8007 substance is first cultivated aerobically in a culture

medium in accordance with a conventional method in quantity which can extract the above-mentioned microorganism or AC8007 substance. As a culture medium illustrated by this invention, AC8007 matter-production bacillus belonging to the above-mentioned Arthrobacter group, Per [ionic exchange pure water 1l], 10 ml of isopropyl alcohol, the yeast extract 0.3g, The peptone 3.0g, the ammonium nitrate 3.0g, the potassium phosphate 0.4g, 1.5 g of phosphoric acid disodium, the magnesium sulfate 0.5g, 10 mg of manganese sulfate, What is necessary is to carry out inoculation to a 500ml Erlenmeyer flask which accommodated 100 ml of sterilized culture media containing 10 mg of sulfate of zinc, cupric nitrate 50mug, molybdenum-trioxide 10mug, and the calcium carbonate 5.0g, and just to carry out shaking culture for three days at 30 **. What is necessary is to carry out 1-ml inoculation of this culture to a 500ml Erlenmeyer flask containing 100 ml of the same culture media as the above, and just to carry out shaking culture to it for five days at 30 **.

[0034]Thus, in order to extract AC8007 substance from an obtained culture, What is necessary is to filter a culture, for example, to add a nonaqueous solubility organic solvent, for example, ethyl acetate, butanol, butyl acetate, etc. to the filtrate, to extract by acid pH, to **** by alkali pH subsequently to water, and just to carry out solvent extraction by acid pH further, since AC8007 substance mainly exists in culture filtrate. This is further given to chromatography by silica gel, alumina, a synthetic adsorbent material, etc., separation generation can be carried out or separation acquisition can also be carried out using high performance liquid chromatography etc. AC8007 obtained substance can also be made into salts, such as alkaline earth metal salt, such as alkali metal salt, such as sodium salt, calcium salt, and magnesium salt, ammonium salt, and a salt with publicly known nontoxic organic amine, by a publicly known method. Thus, AC8007 obtained substance has the physicochemical property which was described above. AC8007 substance of this invention has the operation shown below.

[0035](1) The 0.05-ml intracutaneous vaccination of sarcoma-180 (1×10^8 cells/ml) was carried out behind [one] the ICR mouse 20g (male) of five photosensitization curative effect groups to antitumor action 1Sarcoma-180. Two days afterward, AC8007 substance and hematoporphyrin (HpD; made by a sigma company) were dissolved in a physiological saline which added 3 ml of 0.1mM trischloride buffer solution (pH 7.4) for 15 mg, respectively, and it medicated mouse intraperitoneal with 0.2 ml.

[0036]It is ***** so that heat may not get across an optical exposure to a tumor site for 10 minutes by lumina ace L-150S (product made from a wood clock) which performed pentobarbital anesthesia 10 minutes afterward, and also uses a halogen lamp (JR15V150WB) 10 minutes afterward. A major axis and a minor axis of a tumor were measured from the 3rd, and a value calculated based on the following formulas was made into a size of a tumor.

長い径 (mm) × 短径 (mm)

腫瘍の大きさ=—————

2

After 50 mg/kg intraperitoneal injection, result AC8007 substance controlled growth of prominent sarcoma-180 as by irradiating with light in 20 minutes showed it to Table 1 and drawing 5.

[0037]

[Table 1]

	腫瘍の大きさ (mm)						
	日数 (日)	3	4	5	6	7	8
対 照	非照射	20.1	26.0	35.6	48.3	56.3	52.9
	照射	21.5	27.0	36.0	48.0	58.2	58.1
AC 8007 物質 50 mg/kg	非照射	21.1	28.2	38.1	46.6	57.6	58.1
	照射	21.3	10.6	14.6	17.3	23.9	23.7
ヘマトポルフィ リン 50 mg/kg	非照射	21.0	26.6	37.1	45.6	53.0	53.0
	照射	20.6	17.6	23.3	31.9	60.0	45.5

[0038]2) By the same method as the operation above 1 to Sarcoma-180, one groups [three] used an ICR mouse. AC8007 substance, hematoporphyrin dihydrochloride (NO.H-1875 and about 75% of purity) Sigma company make, hematoporphyrin (NO.H-5518 and about 50% of purity) 5 mg/2 ml, 2.5 mg/2 ml, and 1.25 mg/2ml solution were prepared with a physiological saline which added 0.1M trischloride buffer solution (pH 7.4) so that it might become 25 mg/kg, 12.5 mg/kg, and 6.3 mg/kg about sigma company make, respectively. Intraperitoneal [of a mouse] is medicated with 0.2 ml of this mixed solution, and it is a curative effect ** poor ** A result is as being shown in Table 2 and drawing 6, and it was checked that AC8007 substance excels in an effect hematoporphyrin and hematoporphyrin 2 and HCl used as a control drug.

[0039]

[Table 2]

投与量 (mg/kg)	腫瘍の大きさ (mm ²)	日 数				
		3	4	5	6	7
		0	18.7	22.7	34.1	36.7
AC 800 7 物質	25		7.8	10.5	14.5	17.6
	12.5		11.3	14.4	21.0	23.7
	6.3		19.3	20.9	30.9	34.4
ヘマトポル フィリン 2 HCl	25	12.0	12.8	15.3	24.2	25.1
	12.5		12.9	16.3	25.6	32.0
	6.3		17.4	24.6	35.9	35.8
ヘマトポル フィリン	25		9.7	12.5	20.8	20.0
	12.5		16.1	18.0	28.4	28.9
	6.3		18.1	20.7	39.0	36.7

[0040](2) The 0.05-ml intracutaneous vaccination of Sarcoma-180 (1×10^8 cells/ml) was carried out behind [one] the application ICR mouse to tumor diagnosis. If AC8007 substance 50 mg/kg administration is carried out and it glares through lightguide in two days, the tumor can perform partial determination according to fluorescence.

[0041](3) The 0.05-ml intracutaneous vaccination of the B-16 melanoma (2×10^7 cells/ml) was carried out behind [one] the BDF₁ mouse 20g (male) of three photosensitization curative effect groups to B-antitumor action 1 16 melanoma. AC8007 substance and hematoporphyrin (HPD; made by a sigma company) in seven days, respectively 50 mg/kg, 10 mg/2 ml, 5 mg/2 ml, and 2.5 mg/2ml solution were dissolved in the physiological saline which added 0.1M trischloride buffer solution (pH 7.4) so that it might become 25mg [kg] /and 12.5 mg/kg, and it medicated mouse intraperitoneal with 0.2 ml. Pentobarbital anesthesia was performed 10 minutes afterward, and also it carried out so that heat might not get across an optical exposure to a tumor site for 10 minutes by lumina ace L-150S (product made from a wood clock) which uses a halogen lamp (JR15V150WB) 10 minutes afterward. The major axis and minor axis of a tumor were measured from the 8th, and the value calculated based on the following formulas was made into the size of a tumor.

長径 (mm) × 短径 (mm)

腫瘍の大きさ=_____

2

[0042]After 50 mg/kg and 25 mg/kg intraperitoneal injection, result AC8007 substance controlled growth of B-16 prominent melanoma as by irradiating with light in 20 minutes showed it to Table 3 and drawing 8, and the photosensitization therapy by the optical exposure of HpD was shown in Table 3 and drawing 9.

[0043]

[Table 3]

投与量 (mg/kg)	腫瘍の大きさ (mm ²)						
	日 数						
	7	8	9	10	11	12	
対照	0	11.9	15.9	22.6	27.04	27.46	40.8
AC800 7物質	50	11.7	6.9	13.1	14.4	18.2	20.2
	25	11.5	10.9	14.2	19.8	23.1	30.9
	12.5	11.0	12.2	17.4	21.7	28.1	32.5
H p D	50	11.3	11.10	13.3	15.0	18.0	24.9
	25	12.8	11.3	14.6	21.0	25.4	28.5
	12.5	12.1	11.2	17.3	25.2	28.9	43.3

[0044]As opposed to the tumorigenesis mouse using Sarcoma-180 of a more than, and B-16 melanoma, A curative effect is accepted by PDT and AC8007 substance is accepted to be effective also to the Homo sapiens origin tumors, such as Homo sapiens lung origin malignant tumor A549

share, 521 shares of Homo sapiens large intestine origin malignant tumors AZ, Homo sapiens melanoma G361 share, a Homo sapiens uterine cervix origin Hela cell.

[0045](3) Even if it carried out 400 mg/kg intraperitoneal injection of the acute toxicity AC8007 substance at the mouse, the example of death was not seen.

(4) A mouse acuteness phototoxic experiment photosensitizer is incorporated into a living body, and if it hits direct sunlight, it will cause photosensitivity. Condition starts in the erythema and pain and itch of HIFU at first, an edema occurs after that, and a necrosis of HIFU is observed although the swelling after several days pulls. When critical, there is also an example which will be in a coma and dies.

1) It injected intraperitoneally so that it might become an experimental method ICR (**, 22-25g) mouse (one groups [three]) with 100 mg/kg and 50 mg/kg about each about AC8007 and HpD. It glared from the upper part of immediately after administration to the mouse for 2 hours (it maintains to 25-28 ** temperature conditions) with the halogen lamp (the lumina ace, Wood Clock, JCR15V, 150WB). At this time, it was 32000 luxs. It usually bred after that and mouse weight and life and death were observed.

2) an experiment result -- the result is shown in Table 4.

[0046]

[Table 4]

群	例 数	死 亡 例		
		1日目	2日目	3日目
コントロール 照射	3	0	0	0
AC 8 0 0 7 100 mg/kg (ip) 照射	3	0	0	0
物質 50 mg/kg (ip) 照射	3	0	0	0
HpD 100 mg/kg (ip) 照射	3	3		
50 mg/kg (ip) 照射	3	1	0	0

[0047]By AC8007 substance (100 mg/kg, 50 mg/kg) administration group, the example of death was not accepted as shown in the above-mentioned table 4. It died from the HpD100 mg/kg administration group by all the days following an example, and 1/3 example of death was accepted by 50 mg/kg administration group on the next day. It is as a weight change being shown in drawing 10, and, as for AC8007 substance group, the increase in weight was accepted like the unprescribed a medicine for the patient exposure control group. It survived by the HpD50 mg/kg administration group, and the loss weight was accepted to following **** and the weight change of 2/3 example was recovered 72 hours afterward on the next day. In acute phototoxicity, AC8007 substance accepted high safety compared with HpD as mentioned above.

[0048]As stated above, dissolve AC8007 substance in the included physiological saline (pH 7.4), and 0.1M trichloride buffer solution by intraperitoneal injection, intravenous administration, internal use, etc. By prescribing this substance for the patient, it goes to a tumor site and a ***** period is irradiated with light, laser, an ultrasonic wave, X-rays, etc. As a result, it can close, if a malignant tumor cell is in a necrosis, and growth of a malignant tumor can be controlled. Therefore, as a dose of effective substance AC8007 substance of this invention, it is 1 per one-day adult – 10 mg/kg, and as a medication method, it dissolves in the physiological saline which added sterile buffer solution (pH 7.4 neighborhood), and intravenous administration, local administration, or internal use

performs.

[Translation done.]